

**MONTANA FISH, WILDLIFE & PARKS
INTERIM PERFORMANCE REPORT**

STATE: MONTANA
GRANT TITLE: FISH CONSERVATION GENETICIST
SEGMENT: T-29-1
PERIOD: 01/01/2005 through 12/31/2007

Objective

The objective of this project is to hire a fisheries conservation geneticist and lab technician that would provide FWP with consistent genetic expertise and analysis on genetic issues pertaining to conservation planning, management, and recovery of native fish species.

Location

Statewide

Accomplishments

Montana Fish, Wildlife & Parks (FWP) hired a fish geneticist on 8 October 2005. This person has continued employment with the FWP, and has accomplished the tasks in the AFA for grant # T-29-1. Between October 2005 and December 2007, FWP's geneticist processed and analyzed nearly 10,000 *Oncorhynchus* spp. samples to determine hybridization levels.

In addition to processing lab samples, FWP's geneticist has provided valuable input on a variety of fish genetics conservation issues. For example, FWP's geneticist has provided advice to FWP and the Upper Basin Pallid Sturgeon Workgroup regarding genetic management in hatchery propagation, stocking, and captive brood stock management. Advice has also been provided on issues related to introgression between sauger and walleye, stocking channel catfish (see Appendix A), and conservation brood stock management for Arctic grayling (see Appendix B).

FWP's fish geneticist has conducted 2 fish genetics courses for FWP employees, and presented two genetics workshops at FWP's Fisheries Division Meeting in 2005. FWP's fish geneticist is an active and integral member of the Arctic Grayling Restoration Committee, the Cutthroat Trout Technical Committee, and the FWP Brood stock Committee. FWP's geneticist has also participated on two graduate student committees.

Variations

None:

Expenditure Recap

Proposed Funding

	Federal Share SWG Planning (52.2%)	Non-Federal (47.7)	Total
Direct costs	\$143,000.00	\$140,189.00	\$283,189.00
Plus indirect costs At a rate of 17.9%	\$25,597.00	\$0	\$25,597.00
Total	\$168,597.00	\$140,189.00	\$308,786.00

Actual

	Federal Share SWG Planning (52.2%)	Non-Federal (47.7)	Total
Direct costs	\$86,465.95	\$92,586.24	\$179,052.19
Plus indirect costs At a rate of 17.9%	\$14,754.86	\$0	\$ 14,754.86
Total	\$101,220.81	\$92,586.24	\$193,807.05

Non-Federal Details

	Salary and Benefits	Lodging and Travel	Supplies and Equipment	University Overhead waiver	Total
FY06	\$19,517.85	\$1,585.30	\$32,640.22	\$6,820	\$60,527.37
FY07	\$25,914.87			\$3,675	\$29,589.87
FY08				\$2,469	\$ 2,469.00
					\$92,586.24

Appendix A.

**University of Montana Conservation Genetics
Laboratory**

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April 19, 2006

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Ken;

Got a little sick of salmonids so I did a little looking into channel catfish, *Ictalurus punctatus*, population genetics. Actually it appears that not much has been done in this area. Most of the genetics work with channel catfish has involved selection for performance traits such as growth, disease resistance, fertility, seinability, survival, dressing percentage, etc. under captive conditions (e.g. Bondari 1983; Rezk et al. 2003) or in developing a genetic map to aid in the discovery of genes influencing performance traits (e.g. Liu et al. 1999, 2003; Tan et al. 1999). Obviously, this work has been directed by the aquaculture industry.

In terms of population genetics and possible concerns about escapement of Tishomingo channel catfish into the Yellowstone River and possibly the Missouri River, here is what I was able to come up with. First, it appears that the Tishomingo fish may have been established from fish collected from the Red River, Oklahoma possibly as long ago as 1949 (Mickett et al. 2003) but, I can't state this for certain. According to Kerry Graves (Tishomingo National Fish Hatchery, personal communication), once established the Tishomingo fish have been perpetuated with random mating and occasionally fish from other sources have been added to it. As far as he knows no biochemical genetic data are available from this broodstock.

In terms of the genetic characteristics of wild and hatchery channel catfish populations, the most pertinent paper I was able to find is that of Simmons et al. (2006). They used amplified fragment length polymorphisms (AFLP) to compare the genetic characteristics of 14 wild and 17 hatchery populations in Alabama. Of the hatchery populations, eight raised at Auburn University had been maintained by selection; seven for increased growth and one for albinism. The other nine hatchery populations were apparently maintained using random mating. The wild populations sampled were specifically

chosen because they existed in drainages that contained one or more of the hatchery populations that were sampled and, all but one had never been knowingly stocked. Generally, two wild populations were sampled from each drainage. The primary purpose of this study was to determine if there was any evidence of the aquaculture industry having a significant impact on the genetic characteristics of the wild populations because of escapement.

Considering just the wild populations, there was evidence of substantial genetic differences among them. Genetic divergence among the populations accounted for about one-third of the total genetic variation detected. There was also a strong geographic component to the pattern of genetic divergence as populations within drainages appeared to be more similar, but not identical, to each other than they were to populations from other drainages. At a broader scale, there was no apparent geographic structure to the pattern of divergence. These results indicate that at least in Alabama substantial genetic differences do exist among wild channel catfish populations.

In terms of levels of genetic variability, the wild populations tended to be more variable than the hatchery populations. Furthermore, the hatchery populations maintained with random mating tended to be more variable than the selected broodstocks. All of the broodstocks except one share at least a partial common ancestry to the original Auburn and Marion broodstocks. The Auburn and Marion broodstocks also share a common ancestry as fish have periodically been transferred between them and both were at least partially established from the initial Red River, Oklahoma fish collection (Mickett et al. 2003). The other broodstock was established from fish collected from Florida (Mickett et al. 2003). The reduced variability in the broodstocks probably reflects founder effects (random genetic changes caused when one population is established from another that tend to result in a loss of genetic variation) and the selection practiced in some.

When the allele frequencies are compared among the wild and hatchery populations, all of the wild populations except two are clearly more similar among themselves than they are to the hatchery populations. The hatchery populations also tend to form a distinct cluster indicating that they are generally more similar among themselves than they are to most of the wild populations. The two wild samples that did not cluster with the other wild samples were placed in the hatchery group. These two populations, therefore, are clearly genetically more similar to the hatchery populations than they are to the other wild populations. These two samples came from Wheeler Lake, which has been stocked with fish from the Marion broodstock and the Tennessee River below the dam that created Wheeler Lake.

The above results suggest that escapement from hatchery/aquaculture facilities has not significantly affected the genetic characteristics of adjacent wild populations. This is not surprising as it appears these facilities are composed of closed earthen ponds located an appreciable distance from adjacent rivers. Thus, escapement to the rivers is highly unlikely except for possibly Hurricane Katrina or an occasional bird. In contrast, it appears that when channel catfish are stocked into waters containing wild populations

that the stocked fish can significantly influence the genetic characteristics of the wild population and at least those immediately downstream.

Other somewhat pertinent studies are those of Hallerman et al. (1986), Carmicheal et al. (1992), Mickett et al. (2003), and Bondari (1984). Hallerman et al. (1986) obtained allozyme data from five channel catfish broodstocks maintained by random mating, nine broodstocks selected for growth most of which were included in the Simmons et al. (2006) study, and a wild population from the Rio Grande River. With the exception of the Rio Grande broodstock, all the broodstocks share at least a partial common ancestry among themselves and with the initial Red River, Oklahoma collection (Mickett et al. 2003). In general, the selected broodstocks contained less allozyme variation than the randomly maintained broodstocks and the wild fish. There was no apparent difference in the amount of detectable allozyme genetic variation between the randomly maintained broodstocks and the wild fish. In terms of allele frequencies, however, the wild fish were substantially different from the selected and randomly maintained broodstocks. As in the Simmons et al. (2006) study, this indicates that wild populations can be genetically quite different from hatchery broodstocks.

Carmicheal et al. (1992) obtained allozyme data from four broodstocks of channel catfish, three of which had been maintained by selection and were previously analyzed by Hallerman et al. (1986), and a wild population from the Red River, North Dakota. These data indicated that the wild population had less allozyme variation than the broodstocks and again was genetically very different from them.

Using the 20 loci analyzed in common between the Hallerman et al. (1986) and Carmicheal et al. (1992) studies, there are substantial allele frequency differences between the Red River, North Dakota and Rio Grande River populations at *GPI-B** and *mIDHP** (Table 1). Genetic differences between the populations account for about 35% of the total genetic variation detected. Thus, these populations appear to be genetically very different from each other. This is not a surprising result given how geographically distant the populations are from each other and that substantial genetic differences have been observed among channel catfish populations over a much smaller geographic scale (Simmons et al. 2006).

Using AFLP data, Mickett et al. (2003) compared the genetic characteristics of 16 channel catfish broodstocks raised in Alabama. Half of these broodstocks had been maintained by selection for growth (7) or albinism (1) and the other half had been maintained by random mating. All of these broodstocks were included in the study of Simmons et al. (2006). The data indicated substantial genetic differences existed among the broodstocks. Genetic differences among them accounted for about 45% of the total genetic variation detected. The vast majority of this divergence, however, was due to the inclusion of a single broodstock in the data that was established from fish collected from Florida. When this population was excluded from the analysis, genetic differences among the remaining 15 broodstocks accounted for only 18% of the genetic variation detected. As discussed previously, these 15 broodstocks all at least partially share a

recent common ancestry. Overall, therefore, these results suggest that very marked genetic differences can exist among hatchery broodstocks established from different sources. Again, this is not surprising considering the large genetic differences observed among wild populations over both a broad and relatively narrow geographic scale.

Bondari (1984) crossed individuals from a broodstock that had been maintained by selection for growth with wild individuals collected from Illinois. After 40 weeks post hatch, the hybrids between the wild and hatchery fish were on the average 35% lighter and 13% shorter than the hatchery fish under hatchery conditions. Unfortunately, Bondari (1984) was not able to obtain any wild progeny for the experiment as apparently in the absence of hatchery fish the stimulants for spawning in captivity were absent (Bondari's interpretation). These results suggest that hybridization between hatchery and wild fish can certainly change performance attributes at least in the hatchery environment. The amount of change observed in this study, however, was probably exaggerated by the use of a broodstock that had been selected for increased growth.

Overall, the available data clearly indicate that substantial genetic differences can exist among wild channel catfish populations from different river drainages over both broad and narrow geographic scales. This suggests restricted gene flow among fish from different drainages and, that there is a good possibility that wild populations possess local adaptations at least at the drainage scale. The available data also clearly indicate that substantial genetic differences can exist between hatchery channel catfish populations established from different sources and wild populations not contributing to the broodstocks.

Although data are not available from the Tishomingo National Fish Hatchery broodstock, the above results suggest that substantial genetic differences almost certainly exist between the Tishomingo broodstock and wild fish in the Yellowstone River and Missouri River. Thus, interbreeding between Tishomingo fish and wild fish in the Yellowstone River and Missouri River is almost certainly going to significantly alter the genetic characteristics of the latter fish unless interbreeding is extremely rare. In terms of adversely affecting potential local adaptations in Yellowstone River and Missouri River fish, introduction of Tishomingo fish into waters in these drainages, therefore, generally should not be recommended. Specifically considering Lake Elmo, however, potential interbreeding between Tishomingo and wild fish in the Yellowstone River and Missouri River probably is of little concern. The lake does not connect with the Yellowstone River and not unexpectedly in this situation the data of Simmons et al. (2006) indicate that the fish in the lake are not likely to significantly, if at all, influence the genetic characteristics of the wild fish. Stocking, however, should be avoided in connected waters as the data of Simmons et al. (2006) also indicate that in this situation the stocked fish can significantly alter the genetic characteristics of wild populations and the data of Bondari (1984) suggest that this can potentially alter phenotypic traits such as growth that are often correlated with fitness.

Robb Leary

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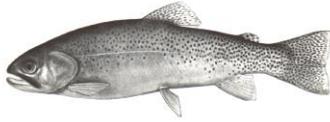
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Table 1

Allele frequencies at the loci showing evidence of genetic variation in samples of channel catfish collected from the Red River, North Dakota and the Rio Grande River, Texas.

Locus	Alleles	Sample and allele frequencies	
		Red River	Rio Grande
<i>GPI-A</i> *	1	0.780	0.357
	2	0.220	0.643
<i>GPI-B</i> *	1	1.000	0.786
	3	--	0.214
<i>mIDHP</i> *	1	--	0.857
	2	1.000	0.143
<i>PGM</i> *	1	0.030	--
	2	0.880	0.929
	3	0.090	0.071

Appendix B.



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December 20, 2005

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Bob;

Following is my review of the draft report **Should fluvial Arctic grayling in the Big Hole River, Montana be considered a distinct population segment (DPS) under the U.S. Endangered Species Act? An evaluation of existing genetic information** (Campton and Ardren 2004).

Genetic variation among Arctic grayling in North America

The first issue addressed was whether or not it seemed appropriate to consider the Arctic grayling, *Thymallus arcticus*, native to the upper Missouri River drainage in Montana a distinct population segment from those native to Alaska and Canada. The conclusion is yes and I agree as this conclusion is strongly supported by the available genetic information.

Using allozyme electrophoretic data, Everett (1986) found marked genetic differences among Arctic grayling collected from the Chena River in Alaska, those descended from fish native to the Athabasca drainage in the Northwest Territories, Canada (Fuse Lake, Montana) and native upper Missouri River drainage populations or populations descended from them. The Athabasca fish had a high frequency of a unique *mIDHP-I*225* allele (form of a gene; 0.353) and a unique *sMDH-A1,2*214* allele (0.179) which strongly differentiated them from all the other samples. With the exception of the Sunnyslope Canal, the Chena River fish were highly divergent from all the other samples as they possessed an unusually low frequency (0.211) of *SOD*145*. The frequency of this allele ranged from 0.500 to 1.000 among the other samples excluding Sunnyslope Canal. The low frequency (0.122) of *SOD*145* in the introduced Sunnyslope Canal fish in the upper Missouri River drainage was attributed to random loss of genetic variation in a population experiencing major fluctuations in size over time rather than indicating an historic affinity to Alaska grayling. The Chena River Fish were also highly divergent

from all the other samples as they were the only ones that possessed a variant *sMDH-B1,2*119* allele (frequency=0.151).

Everett's (1986) data and the presence of a native fish assemblage in Miner Lake in the Big Hole River drainage suggest that Arctic grayling were probably native to the lake. Miner Lake contains burbot and also probably longnose sucker and of all the samples Everett (1986) analyzed it was the only one that possessed the *PGM-1*49* allele. The presence of the *PGM-1*49* allele in the Miner Lake population at high frequency (0.300) and its apparent absence from the native Big Hole River, Madison River, Red Rock Lakes, and Elk Lake populations strongly suggests the Miner Lake population was not solely established from any one or a combination of the other native populations. Thus, the Miner Lake population will be considered to be a native upper Missouri River drainage population in subsequent analyses and discussion.

When the allozyme data of Everett (1986), Hop and Gharrett (1989), and Leary (1990) are combined, information from 21 loci (genes) from the five native upper Missouri River drainage populations, five native populations in the Yukon River drainage in Alaska, and the one population descended from the Athabasca River drainage in Canada are available. Using these data, I partitioned the total amount of electrophoretically detectable allozyme variation into that due to genetic variation within populations (66%), genetic differences among populations within the three drainages (5%), and genetic differences among fish from the three drainages (29%). These results suggest that most of the genetic divergence detected is due to differences among drainages and relatively little is due to differences among populations within a drainage.

Analysis of Arctic grayling mitochondrial DNA (*mtDNA*) using restriction enzymes and DNA sequencing indicated that the fish from the upper Missouri River drainage possessed, in terms of North American fish, an ancestral form of the molecule (different forms of *mtDNA* molecules are referred to as haplotypes) that was generally absent from populations collected from other locations within the species' range in North America (Redenbach and Taylor 1999; Stamford and Taylor 2004). The notable exceptions were that some fish from the lower Peace River drainage Alberta, Canada and the Saskatchewan River drainage Saskatchewan, Canada also possessed this haplotype. The distribution of this haplotype compared to others suggested that Arctic grayling native to the upper Missouri River drainage probably originated from a glacial refuge in the drainage and subsequently migrated northwards when the Missouri River temporarily flowed into the Saskatchewan River. When the Missouri River began to flow southwards the Arctic grayling in the drainage became physically and reproductively isolated from the rest of the species' range.

Considering the allozyme and *mtDNA* data, it is clear that Arctic grayling in the upper Missouri River drainage are highly divergent from those in other portions of the species' range. This is not unexpected given their disjunct distribution and complete reproductive isolation for tens of thousands of years (Redenbach and Taylor 1999). Because of their disjunct distribution and high amounts of detectable genetic divergence, upper Missouri

River Arctic grayling certainly would warrant being considered a distinct population segment under the U.S. Endangered Species Act.

Genetic variation among native upper Missouri River drainage Arctic grayling populations

Campton and Ardren (2004) suggest that based on allozyme analyses and behavioral comparisons that the lacustrine and fluvial life histories of Arctic grayling in the Big Hole River constitute two evolutionarily distinct lineages. I do not think the data strongly support this conclusion.

Although allozyme data from 39 loci are available from the presumed native Big Hole River, Madison River, Miner Lake, Red Rock Lakes, and the now believed extinct Elk Lake populations, only two of the loci analyzed (*GAPDH-3** and *SOD**) were generally variable (polymorphic) among them (Everett 1986; Leary 1990). Thus, in terms of comparing the genetic characteristics of these populations using allozyme data we are really using information from only two loci. When performing population comparisons, therefore, we must keep in mind that we are dealing with a very weak data set. That is, since we are basically using information from only two loci there is apt to be a lot of noise in the data and apparent similarities or differences may be more fortuitous than real. In this situation, results must be interpreted cautiously.

Since the data set basically consists of only two polymorphic loci each with just two alleles, we do not need to estimate genetic distances between samples and subject them to clustering or similar analytical procedures in order to display the apparent similarities or differences among samples. All we need to do is construct a bivariate plot using the frequency of one of the alleles at each locus in each sample. We only need to consider the frequency of one allele at each locus because the frequency of the other allele is just one minus the frequency of the former allele. Analytically the frequency of the other allele, therefore, is redundant.

The bivariate plot of the *GAPDH-3*null* and *SOD*145* allele frequencies indicates that the two genetically most similar samples appear to be the Big Hole River and Madison River (Figure 1). The lake samples, however, do not appear to constitute a separate group. Rather, the Miner Lake population is very divergent from all the others and this divergence would be even greater if we considered the unique *PGM-1*49* allele that was detected in the sample. Apparently, there is also significant divergence between the Red Rock Lakes and Elk Lake samples. The Elk Lake sample appears only slightly more similar to the Red Rock Lakes sample than the Big Hole River and Madison River samples. Thus, the lacustrine samples do not appear to form a genetically distinct group. Taken at face value, therefore, these data do not support the premise that the fluvial and lacustrine life histories fall out into two distinct lineages. In contrast, they suggest relatively substantial divergence among populations regardless of life history.

Redenbach and Taylor (1999) used restriction enzyme analysis of *mtDNA* to compare the genetic characteristics of Arctic grayling from the Big Hole River (N=10), Red Rock Lakes (N=5), and the Madison River (N=10) among themselves and with other populations collected throughout the species' range in North America. They found evidence of only two haplotypes in the upper Missouri River samples and all individuals possessed the same haplotype except a single fish from the Madison River. The paucity of genetic variation detected, therefore, precludes making any inferences about the genetic similarities or differences among the upper Missouri River populations sampled except that they all appear to share a common maternal lineage.

A comparison of rheotactic behavior among age 0⁺ Arctic grayling from the native Big Hole River, Red Rock Lakes, and Madison River/Ennis Reservoir populations as well as introduced populations in Lake Agnes and Deer Lake indicated the existence of adaptive genetic differences among populations (Kaya 1989, 1991; Kaya and Jeanes 1995). The lake inlet spawning Red Rock Lakes, Madison River/Ennis Reservoir, and Agnes Lake fish had, on the average, an increased propensity to move downstream compared to the Big Hole River and Deer Lake fish. The Big Hole River fish, on the average, had a greater tendency to maintain position in a current than all the others. Finally, the outlet spawning Deer Lake fish, on the average, had a stronger tendency to move upstream compared to all the other populations. These behavioral differences were considered adaptive as they would be expected to increase the chances of juveniles finding suitable rearing areas.

From a distinct population segment, I think an important aspect of the above data is that all populations studied exhibited the full range of rheotactic responses. Some individuals maintained position while others moved upstream or downstream. Thus, behaviorally there is substantial overlap among the populations and it is only on the average that they differ. Furthermore, the data suggest that the average behavioral response to current can evolutionarily be quite labile. The Deer Lake fish were probably established from Red Rock Lakes fish. Within a relatively few generations, therefore, their average behavioral response has changed from migrating downstream to migrating upstream. Such evolutionarily labile attributes are not good characters to use to define evolutionary lineages as similarities among populations may represent convergence rather than common ancestry (e. g. Moritz 1994; Wood and Foote 1996).

In summary, I do not think the available genetic data support recognizing the fluvial and lacustrine life history forms of Arctic grayling in the upper Missouri River drainage as distinct population segments. The available protein electrophoretic data is weak and fails to separate the populations into groups based on life history. The *mtDNA* data basically provide no insight into population differences because of the extremely low amount of variability observed. The fact that the three populations analyzed all shared the same haplotype, however, suggests that they all share a fairly recent maternal ancestry. Finally, the behavioral data suggest shared attributes among populations and that average response to current is evolutionarily labile and not suitable for assessing relationships among populations. If Arctic grayling in the United States are to be divided into distinct population segments, the available genetic data suggests the appropriate division would

be Alaska and the upper Missouri River drainage as these have clearly been evolutionarily separate groups of fish for a substantial period of time.

Sincerely,

Robb Leary

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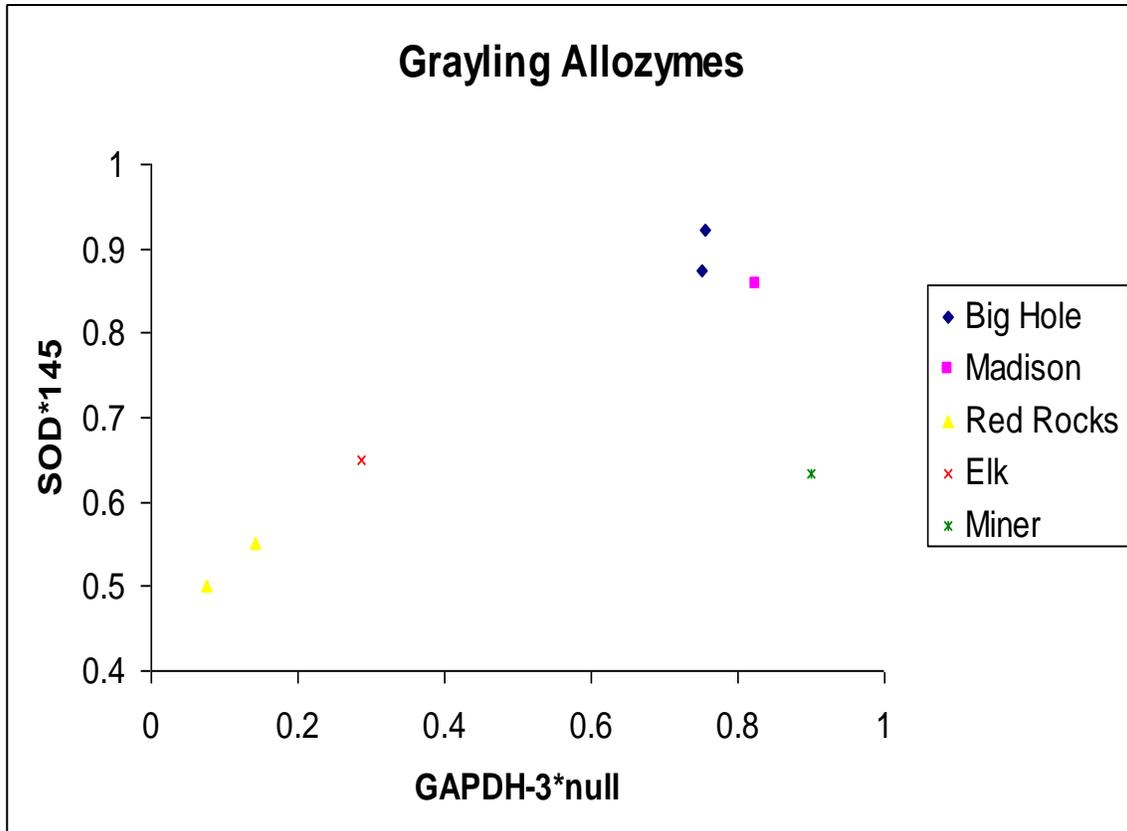


Figure 1. Plot of *GAPDH-3*null* and *SOD*145* allele frequencies in samples from what are suspected to have been five populations of Arctic grayling native to the upper Missouri River drainage, Montana. Note two separate samples were obtained from the Big Hole River and Red Rock Lakes.